The Antibacterial Effects of The Organic Mercurial Compounds

WITH SPECIAL REFERENCE TO THEIR USE AS GERMICIDES FOR THE STERILIZATION OF SURGICAL AND DENTAL INSTRUMENTS

A dissertation submitted to the Board of University Studies of the Johns Hopkins University in conformity with the requirement for the degree of Doctor of Philosophy

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Degree conferred June 1938 Baltimore

Reprinted, with additions, from The Journal of the American Medical Association, May 20, 1939, Vol. 112, pp. 2009-2018

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ACKNOWLEDGMENT

The writer wishes to acknowledge his debt of gratitude to Dr. J. Howard Brown for his help in planning this work, his almost daily guidance, and valuable criticism. He also wishes to express his indebtedness to Dr. Warfield M. Firor for assistance and suggestions relative to the surgical problems involved, and to Dr. Lloyd Felton for his helpful criticism.

Thanks are due Dr. Paul N. Leech, Secretary of the Council on Pharmacy and Chemistry of the American Medical Association, and the various commercial firms for supplying the mercurials tested.

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JOHN H. BREWER, PH.D.

BALTIMORE

PART I

A search of the literature has revealed no carefully controlled work showing that any organic mercurial compound can be relied on to kill the spores of anaerobic bacteria. Nevertheless, claims have been made that these compounds can be depended on for the sterilization of instruments, and vast quantities of them are sold to physicians and dentists for that purpose.

Mercury in its various forms has been used in medicine since the time of the early alchemists, but it remained for Robert Koch [1] to popularize its use as an antiseptic. Immediately after his famous string experiments with anthrax spores the medical world believed that it had in its hands an all powerful germicide which would destroy any disease-producing organism, because apparently Koch had shown that very great dilutions of mercury bichloride would kill the spores of the most resistant organisms. Only a few years after this idea had become thoroughly instilled in the current literature [2] Geppert [3] (1889-1891) with carefully controlled experiments showed that mercury bichloride is a poor germicide although it possesses an almost unbelievable

From the Department of Pathology and Bacteriology, Johns Hopkins University, School of Medicine.

1. Koch, Robert: Zur Untersuchung von pathogenen Organismen, Mitt. a. d. k. Gsndhtsamte. **1**: 1, 1881.

2. Flugge, C.: Mikroorganismen, Leipzig, F. C. W. Vogel, 1886, pp. 542-545.

3. Geppert, J.: Zur Lehre von den Antiseptics, Berl. klin. Wchnschr. **26**: 789, 819, 1889.

antiseptic or, better, bacteriostatic ability. Mercury bichloride at its best possesses several disadvantages which were known to Koch: It corrodes metal instruments, its apparent killing effect is diminished in the presence of organic matter and it coagulates protein. Furthermore, mercury bichloride cannot be used on the skin over long periods of time without causing dermatitis; it is toxic when injected parenterally and therefore cannot be used in chemotherapy. In spite of these objections and the observations of Geppert and others that mercury bichloride cannot be depended on to kill the spores of anthrax bacilli, it continued to be used because of its great antiseptic action. Chemists throughout the world attempted to produce a mercurial compound which would not have these disadvantages and perhaps might kill the sporulating organisms as well. The first products of these researches possessed few advantages over mercury bichloride, but just at the close of the World War several compounds appeared on the open market in the form of organic mercurials. Some of these were apparently free from the disadvantages of mercury bichloride. With certain types of laboratory tests they seem to be more effective than mercury bichloride. Clinical results indicated that they could be relied on as antiseptics. Many claims were made for them, some rightfully and some without the slightest scientific foundation. Among these claims it was suggested that certain of the products could be used for the "cold sterilization" of surgical and dental instruments. In fact, several manufacturers placed on the market preparations especially designed for this purpose. Practically all the germicidal claims made for such products were based on phenol coefficient tests in which only Staphylococcus aureus and Eberthella typhi were used. No adequate tests were made with sporulating organisms such as Bacillus anthracis, Clostridium tetani or the group of organisms causing gangrene.

Claims for the cold sterilization of instruments and for sporicidal activity found their way into many journals, textbooks and standard references. Even as late as 1937 the Council on Pharmacy and Chemistry of the American Medical Association accepted the claim for one organic mercurial that it is "a potent germicide for the spore-bearing and non-spore-bearing

bacteria." [4] No experiments with organic mercurial compounds have been found in the literature comparable to those of Geppert with mercury bichloride.

That sutures cannot be rendered sterile by the use of mercurial compounds was first brought to my attention in 1937 while making a survey of the sterility of catgut sutures for the Council on Pharmacy and Chemistry.[5] Bulloch,[6] Meleney and Chatfield [7] and later Clock [8] pointed out that sutures treated with mercurials are not actually sterile but that the mercurials merely exert a bacteriostatic action on the organisms present. In making this survey for the Council I obtained some sutures which were more than 10 years old. These sutures were "sterilized" by a mercury method and were suspended in 1:1,000 potassium mercuric iodide tubing fluid. When sterility tests were made on them, twelve of the fifty-two sutures were found to contain viable spores, seven of which were of anaerobic bacteria. These results led me to question the advisability of using mercurial compounds for sterilization of instruments, especially after learning that no carefully controlled work had been done in which anaerobic spores were used as the test organism.

DEFINITION OF TERMS DESCRIBING ANTIBACTERIAL ACTION OF THE MERCURIALS

In order to have no misunderstanding regarding the terms used throughout this work, certain words commonly used to describe the antibacterial action of the mercurials are defined.

The present uses of the terms sterile and sterilization are given in a report of the Council on Pharmacy and Chemistry: [9] "The Council on Pharmacy and Chemistry has formally gone on record as disapproving of the use of the terms "sterile," "sterilize" and "steriliza-

4. Merthiolate, New and Nonofficial Remedies, Chicago, American Medical Association, 1937, p. 293.

5. Brewer, J. H.: The Present Status of the Sterility of Catgut Sutures on the American Market, J. A. M. A. **108:** 722-727 (Feb. 27) 1937.

6. Bulloch, William: The Disinfection and Preparation of Catgut for Surgical Purposes, British Medical Research Council Special Report Series, No. 138, 1929, part 1, p. 7.

7. Meleney, F. L., and Chatfield, Mabel: The Sterility of Catgut in Relation to Hospital Infections, Surg., Gynec. & Obst. **52:** 430 (Feb., No. 2A) 1931.

8. Clock, R. O.: The Fallacy of Chemical Sterilization of Surgical Catgut Sutures, Surg., Gynec. & Obst. **56:** 149-161 (Feb.) 1933.

9. Report of the Council on Pharmacy and Chemistry: Use of the Terms "Sterile," "Sterilize" and "Sterilization," J. A. M. A. **107:** 38 (July 4) 1936.

tion" in a bacteriologic sense other than in their correct scientific significance; i. e., meaning the absence or destruction of all micro-organisms. These terms are not relative and to permit their use in a relative sense not only is incorrect but opens the way to abuse and misunderstanding."

The word antiseptic, around which much confusion has arisen in the past, should convey only one meaning: that which is carried in the word itself—the preventing of sepsis or putrefaction.

Regarding the words germicide and germicidal, there has been little difference of opinion among workers. The terms refer to anything that kills germs (micro-organisms).

The words bactericide and bactericidal convey the same meaning as germicide, except that they apply to bacteria only.

The word disinfectant is commonly used to refer to substances used on inanimate objects. The meaning, as the word implies, is to render noninfectious. A disinfectant may be either an antiseptic or a germicide; that is, it may either prevent infection by preventing development of the organisms or destroy them outright.

Bacteriostatic or bacteriostasis refers to an agent or state which prevents the development of the organisms. An antiseptic which does not kill may be a bacteriostatic agent.

For a more detailed discussion of the true value of these words one is referred to Patterson's [10] "Meaning of Antiseptic, Disinfectant and Related Words." I am in thorough agreement with him that if one adheres to the strict denotation of these terms there will be no serious confusion among the laity or professional men.

Much confusion in medical literature has arisen because certain fundamental principles have not been taken into consideration. This is especially true concerning mercurial antiseptics or disinfectants. For instance, mercury bichloride, as Abbott [11] has pointed out, is capable, in strong concentration, of killing Staphylococcus aureus, while in very dilute solutions it is only bacteriostatic. Thus a compound may be anti-

10. Patterson, A. M.: Meaning of "Antiseptic," "Disinfectant" and Related Words, Am. J. Pub. Health **22**: 465 (May) 1932.

11. Abbott, A. C.: Corrosive Sublimate as a Disinfectant Against the Staphylococcus Pyogenes Aureus, Bull. Johns Hopkins Hosp. **2**: 50, 1891.

septic in concentrations too weak to be germicidal. Not only must the concentration of the active agent be considered but the following factors must also be considered in choosing the proper term to describe a particular action: the time of contact, chemical nature of the surrounding medium, the reaction (p_H), temperature, number of organisms present in proportion to the amount of chemical agent, the nature of the organism and the purpose for which the chemical is used.

PRELIMINARY EXPERIMENTS TO SHOW TO WHAT EXTENT ANAEROBES ARE ACTUALLY PRESENT ON INSTRUMENTS AFTER OPERATIONS

As a result of a review of the literature [12] it was decided to determine to what extent anaerobes were actually present on blades after operations. Arrangements were made with the Department of Surgery of the Johns Hopkins Hospital to secure blades used in a series of operations. The nurse in charge of operations had an attendant drop the blades into a sterile tube as the surgeon discarded them. Aseptic precautions were observed and there was very little chance of contamination. The blades from 120 operations were examined and 12 per cent were found to be contaminated with anaerobic spore-forming bacteria. Had these blades been "sterilized" by some inefficient chemical means and reused, there would have been the possibility of infecting other patients. Eight per cent of the contaminated blades were those used for incision of the skin, and this closely parallels the observations of Roberts, Johnson and Bruckner,[13] who found 8.5 per cent of the knives used for making the cutaneous incision to be contaminated with Clostridium welchii. These observations, together with the reports of infections in the literature, seem to indicate that the spore-forming anaerobes should also be used in determining whether a chemical agent is suitable for disinfecting

12. At this point, owing to lack of space in THE JOURNAL, a section was omitted containing a review of the literature concerning theories of the mechanism of killing by the mercurials, previous work on the antibacterial activity of mercurials and methods of testing their bactericidal action for the sterilization of instruments. A short review of the literature relative to the instances of anaerobic infection traceable directly to faulty sterilization of instruments is also included. The report of these studies, together with a supplementary bibliography, makes up part II of this booklet.

13. Roberts, Kingsley; Johnson, W. W., and Bruckner, Helen S.: The Aseptic Peritoneal Cavity—A Misnomer, Surg., Gynec. & Obst. **57**: 752 (Dec.) 1933.

instruments. For the experiments to follow it was decided that pathogenic anaerobes should be chosen on the basis of their mode of attack on the body and the frequency with which they occur in nature. Clostridium tetani, Clostridium septique and Clostridium welchii were chosen. Clostridium tetani remains localized in the tissues and kills entirely by virtue of its toxin. Clostridium welchii and Clostridium septique invade the tissues. Clostridium septique may be found in large numbers in the pericardial and peritoneal cavities after fatal infection, and these infections are always characterized by dark red muscles and edema. In infections caused by Clostridium welchii the muscles are pale and spongy as the result of gas formation. An important characteristic of the clostridia which is not often appreciated is that all the pathogenic members are able to form spores in the animal body (Topley and Wilson,[14] p. 672).

In order to check the technic used and to make the experiments comparable to those of other workers, several different kinds of bacteria have been used as controls. In certain instances Bacillus anthracis (a spore-forming aerobe), Staphylococcus aureus, Corynebacterium diphtheriae, Escherichia coli and Eberthella typhi were used. These include representatives of both the gram-positive and the gram-negative nonsporulating bacteria.

INTRODUCTION TO METHODS USED FOR TESTING THE MERCURIALS FOR THE STERILIZATION OF INSTRUMENTS

The opinion regarding the evaluation of disinfectants is divided into two more or less distinct groups: those who would place every confidence in the results of some laboratory tests and those who accept only clinical data; that is, whether the agent actually works in practice. A far greater number of physicians fall into the latter class and there is much to be said in favor of such an opinion. Garrod (1935) has well stated: "It is customary to deride laboratory conditions as [being] too far removed from those of practice to afford significant results; the truth is that practical conditions can be imitated closely and in almost every particular. The

14. Topley, W. W. C., and Wilson, G. S.: The Principles of Bacteriology and Immunity, ed. 2, Baltimore, William Wood & Co., 1936.

virtue of a test may in fact be held to depend largely on how far it achieves this, and the whole art of devising it consists in securing the necessary conditions without sacrifice of uniformity."

If one attempts to evaluate chemical agents for use on the skin or mucous membrane, to act as either antiseptics or disinfectants, many factors at once present themselves which tend to make it impossible to reproduce actual uniform conditions. Among these factors tissue permeability, individual variation in the animals, including resistance, drug sensitiveness and many other variants, become apparent if one attempts such tests. In testing these chemical agents for the sterilization of instruments, however, there is no reason why the actual conditions cannot be reproduced to the finest detail. The procedure is an entirely in vitro one with none of the variable elements of in vivo tests entering in. Since this is true there is no reason why the standard method for testing such chemicals should be based on any type of test other than one which exactly reproduces the condition under which the chemical is to be used.

In order to make the proper approach to the problem, it was thought wise to secure some reliable information concerning the actual use of these mercurials in the "cold sterilization" of instruments. Since the larger part of the surgery done by physicians is carried out in hospitals where steam sterilization is accessible, chemical solutions are not used to the extent that they are in the dental field, where very little surgery is done in hospitals. Practically every dental surgeon must provide for the sterilization of his own instruments.

DENTAL QUESTIONNAIRE TO DETERMINE ACTUAL CONDITIONS OF USE OF THE MERCURIALS

Before attempting a study of the antibacterial effects of the organic mercurials used for disinfection of instruments it was necessary to know to what extent these substances were used, the treatment of the instruments before and after attempting sterilization and how long they were left in the solution before being considered safe for use. With the aid of the secretary of the Council on Dental Therapeutics of the American Dental Association, a questionnaire was prepared and sent to all class A and B dental schools in this country

and the following dental clinics: Mayo Clinic, Section on Dental Surgery, Rochester, Minn.; University of Chicago, Zeller Foundation Clinic, Chicago; the Forsyth Dental Infirmary for Children, Boston, and the Murray and Leonie Guggenheim Dental Clinic, New York.

In addition, the questionnaire was sent to a representative group of individual dentists over the country, some with very large practices and others with comparatively few patients. Although only sixty-five questionnaires were sent out, the answers represent a cross section of dental practices, since all of the dental schools are included.

Much thought was given to framing the questions in order that they might be clearly understood and so that the answers might be comparable. In view of the definitions given earlier in this paper, some question arose as to the proper word to be used in connection with the treatment of the instruments. Disinfection more adequately describes the actual accomplishment in most cases, but at the time this questionnaire was circulated practically all the commercial solutions carried the phrase "for the sterilization of instruments." A large number of the publications on the subject deal with the chemical sterilization, or chemical agents used for the sterilization, of instruments. Seventy-one per cent of the dentists advocated the use of chemical agents for the sterilization of some instruments. These were usually cutting instruments, scalpels, nerve broaches, tissue shears or instruments made of rubber or other material which would be damaged by boiling.

Sixty-three per cent of those making use of some chemical agent for sterilization use a mercurial. Metaphen, potassium mercuric iodide, merax, mercresin, mercarbolide and merthiolate were the mercurials used. Almost 50 per cent of those using a mercurial used metaphen germicidal solution (1:2,500).

Two of the factors known to influence the chemical disinfection of instruments are the preliminary treatment the instruments receive and the length of time they are allowed to remain in the solution. According to the replies, the time elapsing after the instrument was used until it was placed in the solution ranged from immediately after use to twenty-four hours or until the instrument was needed. The object of this

question was to determine whether enough time elapsed for the blood and saliva to dry. In those instances the instruments would be much more difficult to disinfect. Practically all of those suggesting the use of any chemical agent thought it advisable to wash the instruments before placing them in the chemical solution. Several scrubbed with a brush or other scrubbing agent and a few used scrubbing with soap.

There was a great variation in the length of time the instruments were left in the solution, the time varying from one minute to four hours. Almost every minute up to thirty was suggested as the minimum before the instruments were considered safe to use. Sixty per cent of those answering this question considered a period between ten and thirty minutes as the minimum time for sterilization. At the completion of the sterilization 45 per cent suggested drying on a sterile towel and placing in cabinets before use. The other treatments suggested were rinses in various sterile solutions, saline solutions, boric acid, distilled water or alcohol and drying in air.

Regardless of the value of a questionnaire as a means of obtaining reliable information, this one served to furnish the names of the mercurials actually being used, fixed the maximum and minimum time limits and stated the condition of the instruments when placed in the solutions. This information was necessary in designing experiments to simulate actual conditions of use.

MERCURIALS USED IN THE EXPERIMENTAL WORK

The list of mercurials to be used in the experimental work was made up of all those mercurials named in the answers to the dental questionnaire together with those mentioned as agents which might be used as antiseptics or disinfectants in the 1937 edition of New and Nonofficial Remedies. Included are a few new ones which have not been submitted to the Council on Pharmacy and Chemistry or at least have not been accepted at the present date. Some of the mercurials, owing to their dye content or for other reasons, are not suitable for use on instruments and are not promoted for such purposes but have been included because they represent different types of the organic mercurial compounds. Several inorganic compounds have been included to act

as controls and some because they are still being employed to a large extent for the disinfection of instruments.

The mercurials used are shown in table 1. The information in this table has been derived from various sources: manufacturers, publications and in some instances consulting chemists. The material will be of value to those unfamiliar with the trade names and to persons wishing to make chemical comparisons.

The chemicals for the most part were secured from the secretary of the Council on Pharmacy and Chemistry. In some cases the products were secured directly from the manufacturer and in others they were purchased on the open market.

Since it is not my purpose in this work to compare the efficiency of the various preparations except as to whether they are capable of accomplishing complete sterilization, no attempt has been made to keep the dilutions of the different mercurials constant. The rule generally followed was to use the dilution suggested for the sterilization of instruments or, if the product was not advocated for this purpose, the dilution used was that mentioned for disinfecting the skin or wounds.

In the case of the proprietary mercurials, the solutions were obtained in original packages, since in several instances other chemicals were incorporated to keep the mercurial in solution, to aid in penetration or to prevent rusting of the instruments. In certain instances they are obtainable only in solution form. The nonproprietary chemicals were made up in accordance with suggested directions for use. With some of these it was necessary to make fresh solutions often; with others, large amounts could be made up and kept in glass stoppered bottles in the dark. In every case more than one lot was tested with each organism.

Although it has been shown by Moll,[15] Joachimoglu [16] and others that the reaction (p_H) greatly affects the bactericidal action of the mercurials on spores, no attempt has been made in the experimental work to adjust the p_H. The stock solutions were used as they came from the manufacturer, since it was desired to

15. Moll, T.: Untersuchungen über die Wirksamkeit einiger chemischer Desinfektionsmittel auf Tetanussporen, Centralbl. f. Bakt. **84:** 416, 1920.

16. Joachimoglu, G.: Ueber den Einfluss der Wasserstoffionenkonzentrationen auf die antiseptische Wirkung des Sublimats, Biochem. Ztschr. **134:** 489-492, 1923.

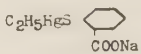
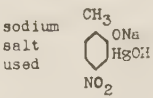
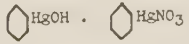
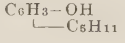
test the solutions as obtainable by the physician. It was assumed that the solutions on the market would be of the optimal p_H for the maximum killing effect. In the few cases in which I made the solutions, unbuffered distilled water was used. The p_H of the various solutions was found to be between 3.5 and 10.5, and stock solutions of the different mercurials varied little from lot to lot.

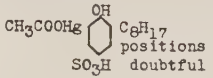
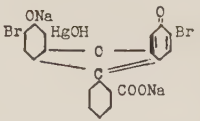
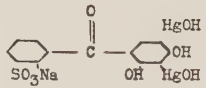
EXPERIMENTAL

Method Used to Obtain Spores for the Experimental Work.—Before I began the actual experiments it was necessary for me to have spore suspensions of the various anaerobes in pure culture. One serious source of error in testing disinfectants with spore-forming organisms has been that the method of cultivation before the test has not been conducive to spore formation, with the result that the tests have simply been made on vegetative forms which were no more resistant than staphylococci or other vegetative organisms. An interesting example of this has been found in securing information on the organic mercurials from the manufacturers. A letter was sent to each of the manufacturers of mercurials asking for information on their product, with special regard to its efficiency as an agent for sterilizing instruments. One of the manufacturers in answer wrote a letter including protocols of experiments showing that its particular product was very effective for killing Clostridium welchii. The exact words were " . . . is very effective for the sterilization of instruments. This statement is based on experiments which showed that surgical instruments contaminated with blood and three representative organisms such as Staph. aureus, B. coli and the spore-bearing B. welchii were sterilized in 5 minutes or less."

On close examination of the protocols it was suspected that the organisms had been grown in beef infusion broth containing dextrose in order to get a heavy suspension for the test. Clostridium welchii does not form spores in the presence of this carbohydrate.[14] For confirmation, a culture of the strain used was obtained from the company and cultural tests showed no spores to be formed in the medium as used by the manufacturer. Thus it was necessary before the experi-

TABLE 1.—*Mercurials Used in the Experimental Work*

Name	Manufacturer	Chemical Name	Mercury Content	Structural Formula*	Empirical Formula	Type	Comment	p_H	Concentration of Type Used
Merthiolate	Eli Lilly & Co.	Sodium salt of ethyl mercuri thiosalicylic acid	49%		$C_9H_9O_2S\ Hg\ Na$	$R_1Hg\ S\ R_2$	Where R_1 and R_2 are different organic radicals	9.8	1:1,000 isotonic solution
Metaphen	Abbott Laboratories	Anhydride of 4,nitro-3-hydroxy mercuri ortho cresol	56-57%		$C_7H_5O_4Hg\ N$	R Hg X	.....	10.5	1:2,500 aqueous germicidal solution
Merphenyl nitrate	The Hamilton Laboratories	Phenyl mercuric nitrate (basic)	63%		C_6H_5HgOH, $C_6H_5HgNO_3$	R Hg X	Where R is an organic and X is an inorganic radical	4.2	1:1,500 boric acid solution
Mercarbolide	The Upjohn Co.	Ortho hydroxy phenyl mercury bichloride	60.95%		$C_6H_5O\ HgCl$	R Hg X	....	6.5	1:1,000 aqueous solution
Mercresin	The Upjohn Co.	Not a chemical entity		A mixture of mercarbolide and five isomeric amyl ortho cresols			....	5.4	Tincture (only form available)

Mertoxol	McKesson & Robbins, Inc.	Acetoxy mercuri 2-ethyl-hexyl-phenol sulfonic acid	40%		$C_{16}H_{24}O_6S\,Hg$	$R_1Hg\,O\,R_2$	.....	3.5	1:1,000 isotonic solution
Mercurochrome	Hynson, Westcott & Dunning	Sodium salt of di-brom hydroxy mercuri fluorescein	24-26%		$C_{10}H_8O_5Br_2HgNa_2$	$R_1Hg\,X$	.....	9.1	1:50 aqueous solution
Meroxyl	Hynson, Westcott & Dunning	Sodium salt of 2-4 di hydroxy-3-5 di hydroxy-mercury benzo-phenone 2′ sulfonic acid	26-29%		$C_{13}H_9O_8Hg_2SNa$	$R_1\begin{matrix}HgX\\HgX\end{matrix}$	.....	7.1	1:200 stabilized aqueous
Mercury oxycyanide	Nonproprietary	.....	85.5%	.....	$Hg(CN)_2.HgO$	Inorganic	A basic mercuric salt of hydrocyanic acid	9.1	1:6,000 aqueous
Mercuric cyanide	Nonproprietary	.....	83.3%	.....	$Hg(CN)_2$	Inorganic		6.2	1:1,000 aqueous
Potassium mercuric iodide	Nonproprietary	.....	25.5%	.....	$K_2Hg\,I_4$	Inorganic	A complex salt formed from 1 mole of HgI_2 and 2 moles of KI	9.2	1:1,000 aqueous
Mercury chloride	Nonproprietary	Mercuric chloride, "corrosive sublimate"	83.3 %	.....	$HgCl_2$	Inorganic	.....	5.9	1:1,000 aqueous

* The positions of some of the groups are doubtful. For source of information see text.

ments were attempted to have a method of cultivation for each of the anaerobes used to insure adequate spore formation.[17]

Experiments with Dental Burs.—As a result of the information obtained in the dental questionnaire, the following experiments were made. They follow in every detail the methods used by the physician or dentist in "sterilizing" his instruments. These experiments were made to determine whether or not the mercurials under actual conditions of use were capable of sterilizing the instruments when spores of some of the pathogenic anaerobes were used as the test organism. I do not claim originality for this type of test. Practically every hospital in which infection has occurred and the instruments were suspected has called on its bacteriologic laboratory to make just such tests as these. The one object in this work has been to conduct the tests so that in case the organisms had not been killed they would have had the proper conditions for growth.

For the first series of experiments dental burs [18] were sterilized in a Petri dish in the hot air oven. These burs were removed with aseptic precautions and the serrated cutting edges dipped in defibrinated blood to which had been added spores of one of the test organisms. The burs were then placed upright in holes in a rack made to fit inside a Petri dish. The burs were covered by inverting a Spray dish over them. This whole assembly had previously been sterilized in the hot air oven. The transfer and dipping of the burs were accomplished with the aid of a pair of forceps previously sterilized and flamed between transfers.[19] The contaminated burs then represented the contaminated instruments as they came from a patient's mouth, provided there had been loss of blood and the contaminating organism had been present. The burs in the inverted Spray dish were left at room temperature for varying lengths of time before being placed in the chemical disinfectant. One set was washed imme-

17. The technic for obtaining spore suspensions of the several organisms is given in part II of this reprint.

18. Dental burs for this work were furnished by the Ransom and Randolph Company, Toledo, Ohio.

19. Hill, H. B.: Sterilizing Instruments During Bacteriological Autopsy Work, J. Applied Micr. **3**: 964, 1900.

diately (five minutes after being contaminated) and placed in the disinfectant. Sets of burs, that is, one for each organism, were placed in the disinfectant after fifteen minutes, thirty minutes, one hour, four hours and twenty-four hours. This was to determine whether or not it made any difference at what time after use the instruments were placed in the disinfectant. It was thought that the drying of the blood might make mechanical cleansing more difficult and thus affect the sterilization.

Since most of the dentists thought it advisable to wash the instruments and some even to scrub with a brush before placing them in the "germicidal" solution, it was decided to vary this procedure. Some sets of instruments were not rinsed at all, others were rinsed in sterile tap water and still others were scrubbed until apparently clean with a sterile brush and water.

Although the answers to the questionnaire placed the time for sterilization in the chemical solutions from one minute to four hours, it was decided to use as the minimum time ten minutes. This was the minimum time suggested by some of the manufacturers for the sterilization of instruments. In addition, sets of contaminated burs were allowed to remain in the various mercurials for periods of thirty minutes, one hour and twenty-four hours. Immediately after being removed from the sterilizing solutions the burs were placed in 40 cc. of 1 per cent sodium thiosulfate for twenty-four hours to neutralize any of the mercurial which might be present, as suggested by Meleney and Chatfield.[7] The burs were removed from this neutralizer, washed in 40 cc. of distilled water and transferred to infusion broth (40 cc.). They were then incubated in an anaerobe jar and removed when growth was noted. In instances in which growth was found, the organisms were identified to make sure that they were the same with which the bur had originally been contaminated.

The mercurials used were merthiolate 1 : 1,000, metaphen germicidal solution, mercresin and a 1 : 5,000 solution of potassium mercuric iodide (Parke-Davis germicidal disks). This work was done in 1936, and these were the mercurials which were being or had

been used for sterilization of instruments by dentists and dental clinics in Baltimore.

Although this work with dental burs represents seven months of experimental work and sixty complete experiments, a tabulation of the results would be of little value, for in each instance growth was obtained even after twenty-four hours' exposure to the disinfectant when the spores of any of the following organisms were used: Clostridium sporogenes, Clostridium septique, Clostridium tetani, Clostridium welchii and Bacillus anthracis.

The technic in these sixty experiments was varied so that some of the experiments simulated every possible combination suggested or actually used by the dentists.

The mixture of the organisms with blood approaches conditions which would be encountered in surgical practice.

Since in dental practice the instruments are not exposed to sodium thiosulfate, it was decided to repeat some of the experiments and omit this treatment.

The burs were rinsed in disilled water after remaining in the mercurials for one hour and for twenty-four hours respectively. They were placed in broth, and growth of the test organisms was obtained in every instance. Mead [20] found metaphen incapable of killing Bacillus anthracis and Bacillus subtilis spores in one hour. He failed, however, to obtain growth after one and one-half hours. His method was to inoculate agar plates from the instruments after rinsing off the excess germicide. He incubated his cultures only forty-eight hours before making readings. Burke, Sprague and Barnes [21] have shown that this incubation period is not sufficient. Meleney and Chatfield [7] have shown that organisms which have been treated with a disinfectant often do not grow out until the fifteenth day, with a maximum number growing out on the thirteenth day.

It is also to be expected that the practice of placing the instrument in the broth would yield a much higher percentage of positive results than that of simply dipping the instruments into melted agar. In actual practice the instruments might be forced into the tissues,

20. Mead, S. V.: Sterilization for Surgery of the Mouth, Internat. J. Orthodontia **16**: 1097 (Oct.), 1215 (Nov.), 1317 (Dec.) 1930.

21. Burke, V.; Sprague, A., and Barnes, L.: Dormancy in Bacteria, J. Infect. Dis. **36**: 555-560 (June) 1925.

and particles in crevices of the instrument would be removed which might not be dislodged by a simple dipping into agar.

EXPERIMENTS WITH DETACHABLE SURGICAL BLADES [22]

Dental burs with deep grooves and serrated edges were chosen because they presented lodging places for bacteria such as would also be found in joints of hinged instruments. This would make mechanical cleansing more difficult and would tax the germicide to a greater extent. The choice of these rough burs gave no idea what the value of such solutions might be in the case of smooth instruments such as scalpels. For this work detachable surgical blades were used. New blades from which the grease had been removed and those from which it had not been removed were used. These blades were used because they could be submerged in the medium and incubated for the duration of the test. The experiments with these instruments were made along with the dental burs, and in no instance were the results different. That is, growth was obtained in every instance. The conditions of testing and the mercurials used were the same as for the dental burs.

Two possible sources of error in this type of test are to be noted. In these experiments the contaminating blood mixture had been made with defibrinated blood. The contaminating mixture should have been made with whole blood, for the bacteria would then have been entangled in the fibrin and might have been more difficult to remove from the instruments. In actual practice the surgeons' blades are affixed in the handle and many crevices are formed which fill with pus, blood and exudate of various kinds, and, if actual conditions were to be approached, the blades should have been affixed to handles. Since positive results were obtained with the smooth blades and defibrinated blood, these factors are minimized; but had the organisms been killed in any instance these would have been very important factors and should be borne in mind in experiments of this type.

After studying the results of the experiments with burs and detachable blades it was realized that, although the experiments reproduced in detail the methods

22. These blades were furnished for this work by the Surgical Blade Division of the American Safety Razor Corporation, Brooklyn.

actually employed in chemically "sterilizing" instruments, certain bacteriologic technic was not above criticism for reasons to be described later, and perhaps a true picture of the ability of the mercurial disinfectants had not been obtained. The technic of a group of experiments was outlined so that the action of the mercurials on the spores of several organisms could be tested. These experiments were designed to determine the action of the mercurials not on spores entrapped in blood or dried in films in crevices surrounded by protein but naked spores in a large excess of the mercurial. The greatest attention was paid to bacteriologic technic, so that ideal conditions for killing and no chance of accidental contamination would exist.

DEVELOPMENT OF AN EFFICIENT CENTRIFUGE TECHNIC FOR TESTING MERCURIALS

Are the organic mercurials, under ideal conditions, capable of killing or rendering nonviable the spores of anaerobes? With this question in mind the following experimental work was undertaken. The technic was improved from time to time and these improvements are noted in the text.

Ditthorn and Bartel [23] described what they term a more exact method for evaluating disinfectants. Staphylococcus aureus was the only organism tested by these workers, and although *Chemical Abstracts* in reporting this work makes the statement that the authors used the method for "determining the value of the disinfectant in killing spore suspensions," no mention of any sporulating organism could be found in the original publication.

My first experiments using a centrifuge technic were made before I became familiar with the previously mentioned work of Ditthorn and Bartel, but the method used differed in only a few respects. Cultures of Clostridium sporogenes, Clostridium tetani, Clostridium welchii and Bacillus anthracis were grown on agar by previously described methods which insured spore formation. The spores were washed twice with distilled water, which left only a small sediment of bacteria on the bottom of a 15 cc. centrifuge tube. This washing

23. Ditthorn, F., and Bartel, M.: Experiments to Determine a More Exact Method for the Valuation of Disinfectants, Disinfektion **8**: 62-66, 1923; abstr. Chem. Abstr. **18**: 1314, 1924.

was done to get rid of any toxin and soluble organic matter that might have been present. The tubes containing the spores were filled to the 15 cc. mark and, by means of a very fine capillary pipet, the organisms were mixed with the disinfectant. Metaphen germicidal solution and merthiolate were the only mercurials used in these preliminary experiments. The mercurial spore suspension was allowed to stand for fifty minutes and centrifuged ten minutes (one hour total). The disinfectant was decanted and the treated organisms were washed twice. The anaerobic organisms were transferred to cooked meat medium under petrolatum by a technic described by Brown [24] for preventing the organisms from becoming covered with petrolatum and thus preventing growth. The spores of Bacillus anthracis that had been treated by this method were streaked out on agar and planted into serum broth as suggested by Süpfle and Degeler [25] in order to insure growth of anthrax spores which had been treated with disinfectants. All of the cultures in cooked meat and also the serum broth contained viable spores. Since it was possible that the mercurials might have affected the organisms in such a way that although it was possible to recover them in culture mediums they might have no longer been capable of producing disease, guinea pigs were inoculated with 0.5 cc. of the final sediment diluted to 1 cc. at the same time the cooked meat and serum broth were inoculated. Since it was known that the strains of Clostridium welchii and Clostridium sporogenes were nonpathogenic for guinea pigs, these organisms were not used in the animal section of this experiment. One-tenth cc. of 1 : 20 lactic acid was injected along with the tetanus suspension to insure the proper necrosis for infection. The anthrax suspension was injected subcutaneously in the left thigh. The guinea pig inoculated with the untreated anthrax suspension (control) died on the second day; the metaphen and merthiolate treated spores caused death on the third day. The tetanus control and the two animals injected with treated organisms all died on the fourth day after injection.

24. Brown, J. H.: The Vaseline Tube and Syringe Method of Micro Gas Analysis of Bacterial Cultures, J. Exper. Med. **35**: 667 (May) 1922.

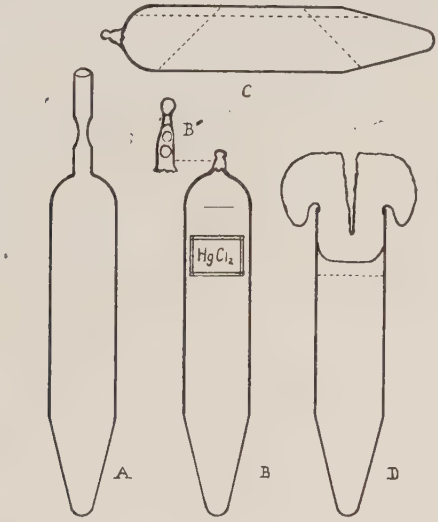
25. Süpfle, K., and Degeler, A.: Die Bedeutung optimaler Nährböden zur Nachkultur bei der Prüfung von Desinfektionsverfahren, Arch. f. Hyg. **85**: 189, 1916.

At this point a critical study of the technic was made and several errors were found. No technic is satisfactory in which there is a possibility that some of the organisms or spores may escape the action of the disinfectant entirely or for even a part of the supposed period of action. In suspension experiments of any type in which the organisms are placed in the disinfectant by means of a pipet, or in the case of the foregoing experiment in which the organisms were already in the centrifuge tube, there are many chances that there will be some bacteria not touched by the chemical agent, or at least not covered by the disinfectant for the full length of the desired period of exposure. This is true even though the suspension is made in one tube and transferred to another tube. Although theoretically possible, I doubt whether one can transfer such a suspension without some of it reaching a point on the tube at which it will not be entirely covered for the duration of the test. The "ringing effect" is also to be considered. That is, the rings left on the test tube as the liquid evaporates may leave a ring of bacteria on the surface of the glass well out of reach of the chemical being tested. This is very important in experiments in which the mercurials are left for as long as twenty-four hours, as in the case of some of the previous tests. This ringing is very noticeable in testing tinctures or solutions containing alcohol such as mercresin. No suspension experiment in which this type of mercurial is being tested can be considered correct unless this ringing factor has been taken into consideration.

To eliminate this source of error, some type of tube which could be sealed off in the flame was sought. If the suspension could be sealed in the proper type of tube and rotated in such a manner that the entire surface would be wetted for the period of exposure to the chemical agent, then this error would have been eliminated. After attempting to seal test tubes off in the flame uniformly, I decided that probably some ampule already on the market might be satisfactory. After trying several types of tubes I found the Judd and Simon [26] modification of the Keidel tube. Tube *A* shown in the accompanying illustration offers several advantages. It has a capacity of 50 cc., which allows

26. Judd, C. C. W., and Simon, C. E.: The Vacuum Tube of Keidel, as Applied to Blood Culture Work, J. A. M. A. **64**: 822 (March 6) 1915.

a large volume of disinfectant to be used with an insignificant volume of spores. The neck of the tube is already constricted and ready for sealing in the flame. The tube is so constructed that it has a centrifuge bottom and, by cutting the tube off with a hot wire and inserting a sterile cotton plug from a test tube of the same size, one has an ideal centrifuge tube for washing the bacteria. This eliminates another source of error in that it is not necessary to transfer the



A, Keidel tube (50 cc.) as described by Judd and Simon. *B*, tube *A* sealed off properly and ready to be placed on rocking machine. *B'*, improper way of sealing tube; allows collection of air in neck of tube. *C*, tube *B* in horizontal position as placed on rocking machine. Dotted lines show changing positions of meniscus as tube is rocked, demonstrating that the entire tube is wetted during each cycle of the machine. *D*, tube *B* with top removed. Note divided cotton plug.

organisms from the time they are exposed to the disinfectant until they are planted into the medium. The point (bottom) of the tube is not sufficiently small to prevent free flow when the tube is rotated.

Since apparently none of the mercurials tested in the earlier work were capable of killing the spores on short contact, it was necessary to have a machine to rotate the tubes satisfactorily over long periods of time. An off-center cam arrangement was made so that when the tubes were placed on it horizontally they would be

entirely wetted in going through each cycle. The dotted lines in *C* show the extremes reached by the meniscus in traveling this cycle. The speed of the motor was set so that eighty complete cycles a minute were made. This was fast enough so that there was no chance for drying in any section of the tube. The tubes were rotated for twenty-three hours. About thirty minutes was required to inoculate from eight to twelve tubes and seal them in the flame before placing them on the machine, and thirty minutes was required for the first centrifuging, so that a total of twenty-four hours elapsed from the time the mercurial came in contact with the spores until it was removed.[27]

The spore suspensions of the anaerobes are made to contain approximately 330,000,000 viable spores per cubic centimeter. One cc. of this suspension is placed in the Keidel tube with 40 cc. of the mercurial to be tested and the tube sealed and placed on the rocking machine. After the tubes had been removed from this machine and centrifuged, a filed line was made just above the label, as shown in *B*, and the tubes were opened by removing the top. All the mercurial but about 0.6 cc. can be decanted or pipetted off without disturbing the sediment. The remaining 0.6 cc. is thoroughly mixed with the sediment by means of a pipet, and about 0.2 cc. is removed for animal inoculation. One loop of the spore-mercurial mixture is planted in an appropriate medium and incubated. Forty cc. of sterile distilled water is added and the organisms are resuspended by washing up and down several times with a very fine capillary pipet. The tube is then plugged and centrifuged. This process is repeated three times, making a total of four washings. Although there is practically no turbidity in the final washing, when the sediment is diluted to 0.4 cc. a slight turbidity will remain, indicating the presence of organisms.

The technic in general has been to inoculate the proper recovery medium; that is, a medium which is ideal for the development of the spores if they have not been killed. In addition, mice have been inoculated to see whether or not the spores have been rendered noninfectious if they have not been killed. Representa-

27. The complete technic is available in part II. Its great detail was considered inadvisable for a clinical publication of this work, and was condensed here.

tive results are given in table 2 and in most cases the protocols were repeated twice, making three sets of experiments.

The large amount of material in table 2 may appear quite complicated. It is hoped that this short explanation will obviate any difficulties:

As an example, Clostridium septique is considered under mercury bichloride. Spores of Clostridium septique were treated for twenty-four hours with mercury bichloride. The spores still suspended in the mercurial were used as an inoculum and were injected into mice and planted in a culture medium. Column 3 shows that the mice did not die even at the end of fourteen days, indicating that the spores were not capable of producing infection. Column 4 shows that the spores grew out in one day, indicating that the organisms were not killed. The results in column 5 and 6 are the same as those in 3 and 4; the inoculum, however, consisted of spores which had been washed four times to get rid of any mercurial present. The results in column 7 under "inoculum: treated spores in mercurial plus lactic acid" indicate that when the spores were injected along with lactic acid they were capable of producing infection and killing the mice. Column 8 shows that Clostridium septique could be recovered by cultural methods. Columns 9 and 10 show results similar to columns 7 and 8.

COMMENT ON THE CENTRIFUGE EXPERIMENTS

If these mercurials had been bactericidal, it would have been necessary to make many additional tests in order to prove the ability of the organic mercurials to disinfect instruments. Tests in the presence of various body fluids would have been made. Tests for organisms dried in pus, whole blood, saliva, bile or, in fact, any fluid which might be found about the body in operations would have been necessary. These tests would be of little value, since it was shown that the mercurials were unable to kill the spores even in twenty-four hours in the absence of body fluids and other factors which are known to decrease the bactericidal action of the mercurials.

One factor that may have influenced the results of these experiments is the production of sulfides in necrotic tissue. It is well known that sulfides are pro-

TABLE 2.—*Results of Mouse and Cultural Tests When Spores Treated with Mercurials Were Used*

Mercurials Tested	Organisms	Inoculum: Treated Spores in Mercurial				Inoculum: Treated Spores Washed Four Times				Inoculum: Treated Spores in Mercurial + Lactic Acid				Inoculum: Treated Spores Washed Four Times + Lactic Acid			
		Death*	Day	Growth†	Day	Death	Day	Growth	Day	Death	Day	Growth	Day	Death	Day	Growth	Day
Mercury bichloride..... 1:1,000	Clostridium tetani...	+	2	+	2	+	2	+	2	+	2	+	2	+	2	+	2
	Cl. septique.....	—	14	+	1	—	14	+	1	+	1	+	1	+	1	+	1
	Cl. welchii.....	—	30	—	60	—	30	—	60	—	30	—	60	—	30	—	60
	Bacillus anthracis...	—	30	+	1	—	30	+	10								
Mercurochrome..... 1:50	Cl. tetani.....	+	2	+	2	+	2	+	2	+	2	+	2	+	2	+	2
	Cl. septique.....	+	1	+	1	+	1	+	1	+	1	+	1	+	1	+	1
	Cl. welchii.....	—	30	+	1	—	30	+	1	—	30	+	1	—	30	+	1
	B. anthracis.....	+	3	+	1	+	3	+	6								
Merphenyl nitrate..... 1:1,500	Cl. tetani.....	+	1	+	2	+	1	+	2	Tetanus		+	2	Tetanus		+	2
	Cl. septique.....	—	14	+	1	—	14	+	1	+	1	+	1	+	1	+	1
	Cl. welchii.....	—	30	—	60	—	30	—	60	—	30	—	60	—	30	—	60
	B. anthracis.....	+	5	+	6	+	5	+	1								
Metaphen..... 1:2,500	Cl. tetani.....	Tetanus‡		+	2	Tetanus		+	2	+	4	+	2	+	3	+	2
	Cl. septique.....	—	14	+	1	—	14	+	1	+	1	+	1	+	1	+	1
	Cl. welchii.....	—	30	—	60	—	30	—	60	—	30	—	60	—	30	—	60
	B. anthracis.....	—	30	—	60	—	30	—	60								
Merthiolate..... 1:1,000	Cl. tetani.....	+	1	+	2	+	1	+	2	+	2	+	2	+	3	+	2
	Cl. septique.....	+	1	+	1	+	1	+	1	+	1	+	1	+	1	+	1
	Cl. welchii.....	—	30	+	2	—	30	+	2	—	30	+	2	—	30	+	2
	B. anthracis.....	+	4	+	6	+	3	+	1								

Mertoxol.....	Cl. tetani.....	+	3	+	2	+	3	+	2	Tetanus		+	2	Tetanus		+	2
1:1,000	Cl. septique.....	—	14	+	1	—	14	+	1	+	2	+	1	+	2	+	1
	Cl. welchii.....	—	30	—	60	—	30	—	60	—	30	—	60	—	30	—	60
	B. anthracis.....	—	30	—	60	+	13	+	1								
Mercarbolide.....	Cl. tetani.....	+	1	+	2	+	1	+	2	+	2	+	2	+	3	+	2
1:1,000	Cl. septique.....	—	14	+	1	—	14	+	1	+	2	+	1	+	1	+	1
	Cl. welchii.....	—	30	+	24	—	30	—	60	—	30	—	60	—	30	—	60
	B. anthracis.....	—	30	+	1	—	30	+	1								
Potassium mercuric	Cl. tetani.....	+	1	+	2	+	2	+	2	+	2	+	2	+	2	+	2
iodide, 1:1,000	Cl. septique.....	—	14	+	1	—	14	+	1	+	1	+	1	+	1	+	1
	Cl. welchii.....	—	30	+	2	—	30	+	2	—	30	+	2	—	30	+	2
	B. anthracis.....	—	30	+	1	—	30	+	1								
Meroxyl.....	Cl. tetani.....	+	1	+	2	+	1	+	2	+	1	+	2	+	2	+	2
1:200	Cl. septique.....	+	1	+	1	+	1	+	1	+	1	+	1	+	1	+	1
	Cl. welchii.....	—	30	+	2	—	30	+	2	—	30	+	1	—	30	+	1
	B. anthracis.....	+	2	+	1	+	2	+	1								
Mercury cyanide.....	Cl. tetani.....	+	1	+	2	+	1	+	2	+	1	+	2	+	2	+	2
1:1,000	Cl. septique.....	—	14	+	1	—	14	+	1	+	1	+	1	+	1	+	1
	Cl. welchii.....	—	30	+	2	—	30	+	2	—	30	+	2	—	30	+	2
	B. anthracis.....	—	30	+	1	-	30	+	1								

* + indicates not only death of the mouse but also characteristic autopsy results and positive cultures from the animal.

† + indicates not only growth but also identification of the organisms inoculated. The number indicates the day on which growth was first observed.

‡ In these instances the animals developed typical symptoms of tetanus but recovered.

duced in decomposing tissue. Whether ammonium sulfide or hydrogen sulfide, it would tend to neutralize the mercurials on or about the spores. This will account for certain instances, not recorded in table 2, in which the animals died but no growth was obtained in the corresponding cultural experiments. The sulfide content of perspiration should be considered in experiments with mercurials. A polished coin will turn dark after being handled, owing to the action of sulfide from the skin. This may account for the failure of the mercurials to disinfect the skin in certain tests. Not all these mercurials are affected immediately by sulfides, but as the mercury ionizes the inactive sulfide is formed.

Topley and Wilson [14] (p. 698) state that the washed spores of Clostridium tetani are innocuous. Vaillard and Vincent [28] demonstrated that many millions of spores, when deprived of their toxin, could be introduced intramuscularly into guinea pigs without producing tetanus. Vaillard and Vincent found that if the tissues are injured in any way the spores will develop and tetanus will occur. A 22 gage needle was used for the injection in the present work and the mice usually struggled. This may account for tissue injury and subsequent tetanus infection in each instance with the washed spores. To preclude the possibility that the deaths were caused by the presence of a small quantity of tetanus toxin, as a control experiment some of the washed spores were further washed in an abundance of antitoxin and then in water. These were injected in the same manner as in the test, and death resulted. According to Topley and Wilson [14] (p. 698) tetanus toxin in a dose of 0.0001 cc. will kill a mouse. The design of this test makes it impossible for a toxin content of even 1 : 1,000,000,000 to be present. If the mercurial treatment is included, the spores were subjected to seven washings and dilution to 40 cc. It has also been shown by Van Ermengem [29] that salts of the heavy metals destroy some anaerobic toxins.

The observations of Moll [15] have been substantiated; that is, some of the mercurials are capable of affecting tetanus spores so that, although not actually killed, they are incapable of causing the death of the animals

28. Vaillard, L., and Vincent, H.: Contribution à l'étude du tétanos, Ann. Inst. Pasteur **5**: 1-39, 1891.

29. Van Ermengem, E.: Ueber einen neuen anaëroben Bacillus, Ztschr. f. Hyg. **26**: 34, 1897.

injected with them. In some instances it would seem that the washing process plays a greater role than the neutralizer in bringing about the recovery of the treated organisms.

Room temperature was used for all the experiments; it varied from 20 to 25 C. No experiments were made to determine the effect of a higher temperature for this is about average room temperature and these agents for "cold sterilization" are usually employed to avoid heating the instruments.

The twenty-four hour treatment period is about as long as could be conveniently employed and is much longer than is customarily used. A chemical agent which would require a longer period would not be practical, for many instruments are expensive and must be sterilized and reused several times in one day.

It is to be reiterated at this point that these experiments were not simply exposure experiments, made according to phenol coefficient methods or the string and bead technic, but were tests in which the organisms were sealed in with the disinfectant and constantly agitated to insure exposure of every organism to the disinfectant for the full period of twenty-four hours. The bacteriologic technic was designed to give accurate information on the one question Are the newer organic mercurials capable of killing the spores of anaerobes under the optimal conditions employed for sterilizing instruments? The answer, evident from a study of the tables, definitely indicates that none of the mercurials tested can be depended on to sterilize instruments in twenty-four hours even in the absence of organic matter and other factors known to decrease the activity of the mercurials.

SUMMARY

1. A review of the literature shows that claims are made that the various mercurials can be depended on to kill the spore-forming anaerobes.

2. A review of the various methods of testing indicates that in most instances bacteriologic technic has been violated or the tests used were not applicable to spore-forming organisms, so that the results cannot be depended on.

3. In a series of 120 surgical operations, bacteriologic examination of the cutting instruments after use

revealed 12 per cent contaminated with spore-forming anaerobes. Eight per cent of the skin knives were found to be contaminated with Clostridium welchii or similar organisms.

4. A study of the mercurial solutions available, summarized in table 1, indicates that these mercurials are used in dilutions of from 1:50 to 1:6,000 and vary in p_H from 3.5 to 10.5. This work was not made for the purpose of comparing the different mercurials available but rather to determine their efficiency as germicides for sterilizing instruments. One cannot help noticing, however, that the commercial products with the highest and lowest p_H were in general the most active.

5. In a series of sixty experiments with dental burs and detachable surgical blades, none of the mercurials were found capable of sterilizing the instruments in twenty-four hours under actual conditions of use. Clostridium welchii, Clostridium tetani and Clostridium sporogenes were used as the test organisms.

6. In preliminary experiments according to the modified methods of Mueller and of Ditthorn and Bartel sources of error were found.

7. An improved centrifuge technic was developed to determine whether the mercurials under optimal conditions are capable of destroying spores of anaerobes. The outstanding features of this technic are as follows: A sealed tube is used in which the organisms are exposed to the chemical agent for twenty-four hours. During this time the mercurial spore mixture is agitated constantly to prevent evaporation "rings" and to insure complete wetting of the entire inner surface of the tube. Settling or layering of the organisms is also prevented. Animal inoculation as well as cultural tests were used to determine the efficiency of the various mercurials with this technic.

8. Not one of the mercurials was capable of killing the spores of Clostridium tetani or of making them innocuous. The work of Moll was substantiated, for in several instances typical symptoms of tetanus developed in the mice and the mice recovered. Contrary to the common belief, Clostridium tetani could be isolated from the site of injection in all the mice dying from tetanus.

9. In the majority of the cases the mercurials were incapable of killing the spores of Clostridium septique, Clostridium welchii or Bacillus anthracis, even in the absence of body fluids and under conditions optimal for the action of the chemical agents.

10. Cultural experiments indicate that some of the mercurials may affect the spores in such a way that, although not actually killed, they are no longer capable of producing infection.

11. The results of the cultural tests show that results based on a comparatively short incubation period are not reliable since, in some instances, the treated spores developed only after twenty-four days.

CONCLUSION

1. The conflicting reports in the literature regarding the germicidal efficiency of the mercurials may be explained by noting that the methods of testing, in most cases, are not applicable to spore-forming organisms, in particular those tests which do not differentiate between actual killing and bacteriostatic action.

2. It is to be concluded from the results of the tests described in this paper that none of the mercurials examined can be depended on to sterilize instruments when spore-forming organisms are present. The question of disinfection is quite another problem. In the case of Clostridium tetani none of the mercurials rendered these spores noninfectious after twenty-four hours of contact. The spores were still capable of infecting the mice and producing tetanus. The spores of Clostridium septique and Bacillus anthracis, however, could be rendered noninfectious in certain instances. That is, although it was possible to recover in these cases organisms in cultural experiments, they did not produce an infection when injected into animals.

3. To explain the ability of some of the mercurials to act as antiseptics is beyond the scope of this paper. However, their actual germicidal value for vegetative bacteria is questioned. After it was found that the mercurial solutions as commonly used are incapable of killing the spores of the pathogenic anaerobes in twenty-four hours, it was decided to determine their effect on some vegetative bacteria by the centrifuge method. Just as statements are made that some of these

mercurials will kill spore-forming bacteria, some of the manufacturers claim that their particular product will kill vegetative bacteria in ten minutes. One of the mercurials used did not kill Staphylococcus aureus in thirty minutes, although no neutralizers or special recovery methods were used. Other vegetative bacteria were used and the results indicate that some method other than one based on phenol coefficient determination should be used to establish the ability of the mercurials to disinfect instruments.

4. Although it was found that the mercurials as available on the market are not germicidal for the pathogenic anaerobes, it is possible that one of the newer organic solvents, wetting agents or surface tension reductants might affect the spores in such a way that the mercurials would then be able to kill these organisms.

5. The method of testing used in the experimental work is not suggested as a standard method to be used for testing chemical agents for sterilizing instruments but is proposed as a method for determining the action of chemical agents on spores under the most favorable conditions. The object of the method is to eliminate all possible sources of error by insuring the action of the chemical on every spore for the full length of the period of exposure.

PART II

HISTORICAL

THEORIES OF THE MECHANISM OF THE BACTERICIDAL ACTION OF THE MERCURIALS

The most generally accepted idea concerning the disinfecting action of the mercurials is that they precipitate the bacterial protein by combining directly with it to form a mercury-proteinate. This theory came about logically, since mercury bichloride was known to combine directly with the tissues and to coagulate their proteins. Smith,[30] writing on the disinfecting action of metallic salts, states that they prevent sepsis by combining directly with flesh, making a permanent compound and keeping out the contaminating air. As stated earlier, Geppert [3] had suggested that the sublimate made a chemical bond with the cell, which could be broken by converting the mercury to the sulfide. Kronig and Paul [31] pointed out that the bactericidal action was due to the free ions of mercury in the solution and not to the molecular concentration. Various authors have enlarged on this protein precipitation theory, but no additional proof of value has been advanced.

Süpfle and Müller [32] and Engelhardt [33] have attempted to show that sublimate is absorbed on the cortical layer of the bacteria and that, by treating with a stronger absorbent (blood charcoal), the mercurial could be separated from the bacteria so completely that germs apparently dead would renew their growth.

This surface adsorption theory fitted so well into the latter observations of colloidal chemists that Bancroft and Richter [34] attempted to explain chemical disinfec-

30. Smith, R. A.: Disinfectants and Disinfection, Edinburgh, Edmonston and Douglas, 1869.

31. Kronig, B., and Paul, T. L.: Die chemischen Grundlagen der Lehre von der Giftwirkung und Disinfection, Ztschr. f. Hyg. u. Infektionskr. **25**: 1, 1897.

32. Süpfle, K., and Müller, A.: Ueber die Rolle der Adsorption bei der Einwirkung von Sublimat auf Bakterien, Arch. f. Hyg. **89**: 351, 1920.

33. Engelhardt, H.: Action of Mercuric Chloride on Bacteria, Disinfection **7**: 63-65, 81-83, 1922.

34. Bancroft, W. D., and Richter, G.: The Chemistry of Disinfection, J. Phys. Chem. **35**: 511, 1931.

tion as a simple adsorption of a germicide giving a reversible reaction. The analogy seemed probable, but it was found that it did not compare to the common reversible chemical reactions since the addition of a large number of bacteria slowed rather than accelerated the velocity of the disinfection.

A theory which fits in well with our modern conception of cell physiology was first described by Duclaux [35] in his "Traité de microbiologie." He assumed that chemical disinfection was simply a destruction of the enzymes of the bacterial cells and without enzymes the bacteria could not live. In the light of our present knowledge of enzyme chemistry and bacterial physiology, we know that the enzymes may be reversibly precipitated by heavy metal salts. Thus we have an excellent explanation of the fact that organisms apparently killed by a mercurial can be revived with suitable neutralizing agents. This theory, further supported by the work of Isaacs,[36] has been tabulated by Gay.[37] The points pertinent to this present work are that in the case of spores each cell is provided with its maximum enzyme content at the start. According to this theory, the coating of mercury apparently has no effect on the enzyme contained within the spore; thus theoretically the spore could exist indefinitely without being affected. This would serve to explain the survival of the spores in the catgut sutures previously described, although suspended in 3 per cent potassium mercuric iodide for more than ten years.[5] This theory would also explain the necessity for having a medium containing the essential elements for the optimum growth of the organisms, for it has been found that injured cells may grow on one medium and not on another. It may be that the enzyme content of the cell may be destroyed to such an extent that only an easily digested medium can be attacked.

One other theory concerning the mechanism of bactericidal action, although not applicable to all the mercurials, is at least worthy of mention. This theory

35. Duclaux, E.: Traité de microbiologie, Paris, Masson et Cie **3**: 461, 1898.

36. Isaacs, M. L.: Factors Which Influence Tests of Bacterial Survival, J. Bact. **20**: 161, 1930; The Effect of the *p*H of the Survival Test Culture on Results Obtained with a Saprophytic Tubercle Bacillus, ibid., p. 175; A Theory of Disinfection, Science **75**: 46, 1932.

37. Gay, F. P.: Agents of Disease and Host Resistance, Baltimore, Charles C. Thomas Co., 1935.

was suggested by Stark and Montgomery [38] to explain the bactericidal action of merphenyl nitrate (first described by Otto in 1870 as phenyl mercuric nitrate). Later examinations indicate that the product is phenyl mercuric hydroxyl nitrate (basic nitrate). It is suggested that merphenyl nitrate differs from other complex mercury compounds in that the mercury-containing ion is the cation (C_6H_5Hg). Since bacteria usually carry a negative charge, the high bacteriostatic power of this compound is attributed to the positive charge of the mercury-containing ion.

None of the suggested theories is, at present, in a sufficiently well developed state to be accepted as the method by which mercurials kill bacteria, and it seems that it may be possible that all mercurials do not affect bacteria in the same manner. It may be that in certain instances each of these theories or a combination of them is correct for a certain type of mercurial.

METHODS OF TESTING THE BACTERICIDAL ACTION OF THE MERCURIALS FOR STERILIZATION OF INSTRUMENTS

Volumes could be written on the methods of testing disinfectants. Each worker has suggested his own particular modification and most of these have been used for some of the mercurials. However, if the subject were limited to the methods used for testing the ability of the mercurials to sterilize instruments all the methods would fall into one of two groups: those in which the instruments have been used and some simulation of the actual conditions of use have been approached, or tests which give some inference as to the ability of the chemicals to kill under certain conditions of testing. Most of the information concerning the organic mercurials has been derived by means of the latter method.

It was discovered very early that mercury bichloride was not suitable for sterilization of instruments. Lord Lister (1870) [39] made use of mercuric cyanide, which had little corrosive action on instruments. He depended on the final outcome of his surgical operations, however, as a test of the efficiency of the various chemicals used.

38. Stark, O. K., and Montgomery, Marjorie: A Study of the Bacteriostatic and Germicidal Powers of Merphenyl Nitrate, J. Bact. **29**: 6, 1935.

39. Lister, Joseph: Further Observations on the Cyanide of Zinc and Mercury, the collected papers of Joseph, Baron, Lister, Oxford at the Clarendon Press **2**: 324, 1889.

Countless experiments have been made by purposely contaminating instruments and then subjecting them to the sterilization process used and finally making sterility tests on them to determine the value of the method. An excellent example of this type of testing is given later in discussing the work of Nye and Mallory.[40] If properly carried out, this method of testing will give the most reliable information, for it makes use of the actual materials and conditions of use. Failure to secure reliable results with this method is usually due to faulty recovery methods, and special attention should be given to the bacteriologic technic involved.

Mead (1930) [20] describes very concisely a method which he used to test the efficacy of one of the organic mercurials. Old dental instruments were liberally inoculated with month old cultures of two spore-bearing bacilli. (The cultures were not mixed. Anthrax culture was put on some instruments and Bacillus subtilis on others). The inoculated points were then dipped into blood and dried in the incubator for four hours. One instrument with each type of bacillus was put into each of three types of fluids. At the end of one and one-half hours agar plates were inoculated from the instruments, the excess solution being rinsed off with distilled water. A discussion of his results is given under the section on experiments on dental burs.

Similar technics have been described by the various manufacturers for testing the ability of their particular product to sterilize instruments. One of these methods representative of the group is as follows:

> The instruments selected were forceps because of rough surfaces and joints which would be more difficult to sterilize than a smooth surface instrument. Two types of forceps were used—tissue and artery forceps. In the earlier experiments the jaws of the artery forceps were closed, but later these were held open.
>
> Three species of bacteria were tested; (1) Staphylococcus aureus 209, the same strain as used in testing disinfectants; (2) Bacillus coli 13; (3) Bacillus welchii, a spore-forming anaerobe.
>
> The forceps were wrapped in paper and sterilized by dry heat at 180 C. No attempt was made to be sure that they were clean before sterilization. The unwrapped forceps were immersed for three minutes in defibrinated blood containing

40. Nye, R. N., and Mallory, T. B.: A Note on the Fallacy of Using Alcohol for the Sterilization of Surgical Instruments, Boston M. & S. J. **189**: 561, 1923.

the test organisms in suspension, removed and allowed to dry in a sterile Petri dish for one hour. They were then placed in a jar containing a sufficient amount of germicide to cover them completely and removed after selected time intervals. The forceps were then rinsed in sterile distilled water to remove any excess of the germicide, placed in a tube containing meat extract broth (or meat infusion broth when Bacillus welchii was used), and incubated at 37 C. for forty-eight hours. The time intervals selected for exposure to the germicide were five, ten and fifteen minutes. Observations were made of the presence or absence of growth in the incubated tubes after forty-eight hours and subcultures were then prepared. Controls were carried through the above process except that distilled water was substituted for the germicide.

Another type of testing makes use of a centrifuge technic. This method is valuable in determining the action of the chemical agent on bacteria alone without particular reference to its use for sterilization of instruments. This method, described independently by Mueller and by Ditthorn and Bartel [23] in 1923, is discussed in detail under the section on the development of an efficient centrifuge technic for testing mercurials.

THE USE OF THE PHENOL COEFFICIENT METHOD

In the long list of methods for testing disinfectants it might be said literally that everything from soup to nuts has been employed. Broth has been used for a long while because of its nutritive value, while the nuts were suggested to replace the glass beads of Kronig and Paul [31] so that an organic factor would be brought into play and thereby more nearly approach actual conditions of use. Of all these various methods of testing none has been so popular as the phenol coefficient type of test. There seems to have been some reason why it has been considered desirable to express results in terms of numbers.

McCulloch [41] ingeniously uses the words of Lord Kelvin to introduce his chapter on the comparisons of disinfectants: "If you can measure that of which you speak, and can express it by a number, you know something of your subject; but if you cannot measure it, your knowledge is meager and unsatisfactory." Pringle [42] compared various agents as antiseptics.

41. McCulloch, E. C.: Disinfection and Sterilization, Philadelphia, Lea & Febiger, 1936, p. 187.
42. Pringle, J.: Observations on Diseases of the Army, London, 1750, American Edition, Philadelphia, E. Earle, 1810.

Using sea water as 1, he compared many other solutions to it, giving them numerical values.

Sternberg (1881) [43] was the earliest worker to employ a mixture of disinfectant and culture, and then after a fixed time a transfer of one or two loops was made to suitable medium. His description of this forerunner of the phenol coefficient test is most interesting. In comparing his method with that of Koch (1881) [1] he writes:

> In the writer's experiments, made in 1880 and subsequently, a different method has been adopted. The time has been constant—usually two hours—and the object has been to find a minimum amount of various chemical agents which would destroy the test organisms in this time; and instead of subjecting a few of the test organisms attached to a silk thread to the action of the disinfectant, a certain quantity of recent culture—usually five cubic centimeters—has been mixed with an equal quantity of standard solution of the germicidal agent. Thus five cubic centimeters of a 1:200 solution of carbolic acid would be added to five cubic centimeters of a recent culture of the typhoid bacillus, for example, and after two hours contact one or two oese would be introduced into a suitable nutrient medium to test the question of disinfection.

Rideal and Walker [44] standardized many of the conditions of testing and gave us what was to evolve finally into the present phenol coefficient test. The evolution of the test has been traced by many authors,[45] finally reaching a more or less stable degree in 1931 when it was last described as the Food and Drug Administration method.[46] It is by this test that most of the present information concerning the organic mercurials has been obtained. It makes use of only two organisms, Staphylococcus aureus and Eberthella typhi. With this method, it is possible to employ only two temperatures and all comparisons are made to phenol. I quite agree with Garrod (1935) that it would be very convenient and desirable to express the value of the disinfectants in terms of numbers, but I would place far above this the desire to test the chemicals under actual conditions

43. Sternberg, G. M.: A Manual of Bacteriology, New York, William Wood & Co., 1892, p. 159.

44. Rideal, S., and Walker, J. T. A.: The Standardization of Disinfectants, J. Roy. Sanitary Inst. **24:** 424, 1903.

45. Tilley, F. W.: Phenol Coefficients, Am. J. Pub. Health **11:** 513-519, 1921.

46. Ruehle, G. L. A., and Brewer, C. M.: United States Food and Drug Administration Methods of Testing Antiseptics and Disinfectants, United States Department of Agriculture, Circular 198, 1931, p. 7.

of use. It is this tendency to express in terms of a numerical coefficient that has led to much of the confusion existing concerning the organic mercurials.

Although Staphylococcus aureus is probably one of the organisms most frequently encountered in the disinfection of instruments, it is by no means the most difficult to kill, and if one selects members of the genus Clostridium, the phenol coefficient method is not applicable. This is only one of the reasons why this method is not suitable for testing chemical agents for disinfection of instruments. Much confusion would have been avoided if this test had never been suggested for use with mercurial compounds. The test as described by Ruehle and Brewer [46] is suggested for these agents with the following stipulations:

> There are certain types of germicidal agents, such as many of the mercury compounds, which give very high results by phenol coefficient tests. Due to the high inhibitory value of such substances in preventing growth in the subcultures these figures are frequently misleading. For germicides used in the disinfection of such objects as surgical instruments, this is of particular importance and must be taken into account. Failure to appreciate this characteristic of certain compounds is much more likely to lead to error when Staphylococcus aureus is used rather than Eberthella typhi as the test organism. That false values may not be obtained for products of this type, or for any other disinfectant giving suspiciously high results, the subcultures should contain very large amounts of medium (not less than 200 cc.) or they should be retransferred by carrying at least four loopfuls from the first subculture to a second tube of broth, as recommended by Shippen (1928).

Shippen's [47] actual conclusion is as follows: "This method for testing germicides, employed in the Department of Agriculture, is not applicable to preparations with high bacteriostatic power, unless modified by the addition of subtransfers." Without detracting from Shippen's excellent work in pointing out the fallacy of using the phenol coefficient test (in its present form) for mercurial germicides, due credit should be given Sternberg [43] for recognizing that in the testing of certain chemicals a retransfer should be made to a second tube of nutritive medium. After discussing the work of Geppert by which the inhibiting sublimate is precipitated as an inert sulfide, Sternberg, in discussing

47. Shippen, L. P.: A Fallacy in the Standard Methods of Examining Disinfectants, Am. J. Pub. Health **18**: 1231, 1928.

his previously mentioned method, counseled that "with most agents simple dilution will serve the purpose of preventing an erroneous inference from the restraining influence of the chemical agent being tested. If we carry, by means of a platinum loop, one or two öse into five to ten cubic centimeters of bouillon, the dilution still further to be on the side of safety, by inoculating a second tube containing the same amount of sterile bouillon from the first, carrying over in the same way one or two öse." Shippen gives full credit to Abbott [11] for the excellent discovery that the amount of mercurial carried over by a loop is sufficient to produce bacteriostasis of the treated organisms, but Abbott does not mention a remedy except generally, and he did not make the suggested remedy as did Sternberg of retransferring one or two loopfuls to a second tube of broth.

It is interesting to note that, although the method used by the department of agriculture was published in 1931 and Shippen's paper in 1928, there have been only three published papers in which this retransfer test [48] has been used. However, the manufacturers of mercurial germicides for the disinfection of instruments list an enormous number of references, giving phenol coefficient or "killing" dilutions according to phenol coefficient methods.

PREVIOUS WORK ON THE ANTIBACTERIAL ACTIVITY OF THE MERCURIALS

Chemical Abstracts list more than 220 references to the antibacterial effect of mercury bichloride alone. An equally large number has been found concerning the organic mercurials. References will be made only to those in which the spore bearers have been used as the test organisms or some statement has been made regarding the use of organic mercurials for sterilization of instruments.

The earlier claims made by Koch for mercury bichloride have already been discussed. Seymour-Jones [49] published an anthrax sterilization method which made use of corrosive sublimate. Proof that mercury bichloride was not satisfactory for killing spores has

48. This was first brought to the author's attention by Mr. E. B. Carter, Abbott Research Laboratories, North Chicago, Illinois.

49. Seymour-Jones: Anthrax Sterilization Method, Bradbury, Agnew & Co., The Leather Trades Reviews, January 1911, as referred to by Gegenbauer: Arch. f. Hyg. **87**: 289, 1918.

been demonstrated by Geppert,[3] Heider,[50] Xylander,[51] Croner and Naumann [52] and Gegenbauer (1918).[53]

More recently Moll [15] demonstrated with the aid of animals and cultural experiments that mercury bichloride could not be depended on to kill tetanus spores. Tizzoni and Cattani [54] had found mercury bichloride unsatisfactory for killing Clostridium tetani, but one cannot make sure that spores were present in their experiments.

Reports on the ability of the other nonproprietary mercurials vary from favorable, when nonsporulating organisms were used, to attempts to show that they are no more active than mercury bichloride on spores. Mackie (1928) and Bulloch [6] (1929) have shown that mercuric iodide, potassium mercuric iodide and mercuric cyanide were unsatisfactory for sterilizing spores on catgut.

Raiziss and Severac [55] report a series of experiments with metaphen. These experiments indicate that metaphen is eleven times more powerful than mercury bichloride when tested against the spores of Bacillus anthracis, with an exposure of one hour.

Powell and Jamieson [56] state: "The active material in Merthiolate is 40 to 50 times as effective as phenol upon S. aureus and B. typhosus. B. coli, various streptococci, and the tetanus bacillus were all found to be in this general range of susceptibility to Merthiolate."

Birkhaug [57] reports the highest dilution of disinfectant killing Bacillus subtilis after ten minutes, but not after five minutes, contact at 37 C. as follows: phenyl

50. Heider, A.: Ueber die Wirksamkeit der Desinfectionsmittel bei erhöhter Temperatur, Arch. f. Hyg. **15**: 341, 1892.

51. Xylander: Beitrage zur Desinfektion von milzbrandhaltigen Hauten. Arb. a. d. Gsndhtsamte **25**: 457, 1907.

52. Croner, F., and Naumann, C.: Vergleichende Untersuchungen über die Desinfektionswirkung von Sublimate und Sublamin, Deutsche med. Wchnschr. **39**: 1784, 1911.

53. Gegenbauer, Viktor: Ueber das Seymour-Jonessche Sublimat Ameisensäure-Verfahren zur Desinfektion milzbrandiger Felle und Häute, Arch. f. Hyg. **87**: 289, 1918.

54. Tizzoni, G., and Cattani, G.: Ueber die Widerstandsfahigkeit der Tetanusbacillen gegen physikalische und chemische Einwirkungen, Arch. f. exper. Path. u. Pharmakol. **28**: 41, 1891.

55. Raiziss, G. W., and Severac, M.: A New Organic Mercury Compound with Powerful Germicidal Properties, J. Lab. & Clin. Med. **9**: 71, 1923. Raiziss, G. W.; and Severac, M., and Moetsch, J. C.: Bacterial Chemotherapy with Special Reference to Mercury Dyestuffs, J. Pharmacol. & Exper. Therap. **26**: 447, 1926. Raiziss, G. W., and Severac, M.: Metaphen and Its Bactericidal Properties, J. Infect. Dis. **40**: 447-452, 1927.

56. Powell, H. M., and Jamieson, W. A.: Merthiolate as a Germicide, Am. J. Hyg. **13**: 297, 1931.

57. Birkhaug, K. E.: Phenyl-Mercuric-Nitrate, J. Infect Dis. **53**: 250, 1933.

mercuric nitrate 1:65,000, merthiolate 1:24,000, metaphen 1:36,000, mercurochrome 1:300, mercury bichloride 1:12,000.

Simmons [58] reported that mercurochrome would not kill the spores of B. anthracis in ten hours. Scott, Hill and Ellis,[59] however, found B. anthracis to be killed in ten minutes.

Weed and Ecker [60] report that phenyl mercury nitrate kills B. subtilis, Cl. septique, Cl. tetani, Cl. welchii, Cl. histolyticum, Cl. sporogenes and Cl. chauvei in ten minutes or less.

Clock [8] reports that he was unable to sterilize catgut, artificially infected, with a sporulating organism, using potassium mercuric iodide, metaphen, mercurochrome, merthiolate or mercurophen.

McCleskey and Swingle [61] found in using mercresin and mercarbolide that spores of Cl. welchii were not killed after exposure of 120 hours.

Scott [62] gives the following results: metaphen was incapable of sterilizing spores of Cl. chauvei, Cl. septicum, Cl. novyi or B. subtilis. Merthiolate failed to kill Cl. chauvei, Cl. septicum or Cl. novyi in less than three days. Phenyl mercury nitrate failed to sterilize Cl. septicum and Cl. tertium in less than three days.

Ecker and Smith [63] suggest the use of merphenyl nitrate for sterilizing several pieces of surgical equipment. They state that potassium mercuric iodide failed to kill certain organisms in their experimental work.

Although this review of the literature bearing on the ability of the mercurials to kill spores lays no claim to completeness, it is at least representative of the work done in which spores were used.

Most of the results in which strong bactericidal action against spore-forming organisms is reported were obtained by phenol coefficient or similar methods which

58. Simmons, J. S.: Bactericidal Action of Mercurochrome 220, Soluble and Iodine in Skin Disinfection, J. A. M. A. **91**: 704, 1928.

59. Scott, W. W.; Hill, J. H., and Ellis, M. G.: Action of Mercurochrome and Tincture of Iodine in Skin Disinfection, J. A. M. A. **92**: 111, 1929.

60. Weed, L. A., and Ecker, E. E.: The Utility of Phenyl-Mercury Nitrate as a Disinfectant, J. Infect. Dis. **49**: 440, 1931; Bactericidal Action of Phenyl-Mercury Nitrate: Further Observations, ibid. **51**: 309, 1932.

61. McCleskey, C. S., and Swingle, E.: A Comparative Study of the Germicidal Activity of Certain Compounds, Iowa State Coll. J. Sc. **11**: 177-183, 1937.

62. Scott, J. P.: The Action of Preservatives and Salts on Blackleg Cultures, J. Infect. Dis. **61**: 103-109, 1937.

63. Ecker, E. E., and Smith, R.: Sterilizing Surgical Instruments and Utensils, Modern Hosp. **48**: 92, 1937.

do not take inhibition into consideration. In those instances in which recovery of the organism is reported the technic used does not make sure that all organisms were exposed to the disinfectant for the full period of testing.

THE CHOICE OF ORGANISMS TO BE USED IN THE EXPERIMENTS AND THE IMPORTANCE OF CLOSTRIDIA AS AGENTS OF INFECTION

Walters [64] has shown that there is such a wide variation in the specificity of disinfectants for various organisms that the use of a set standard is without value unless several organisms are considered. As stated earlier, many tests have been made on these mercurials using Staphylococcus aureus and Eberthella typhi, but tests in which the sporulating anaerobes were used have been sadly lacking or, if reported, failure to observe bacteriostatic conditions or other errors in technic have been found.

The great resistance of the spores of pathogenic anaerobes to heat and chemical agents suggests them as probably the most important organisms to be considered in determining the value of the organic mercurials as germicides for the disinfection of instruments. Since these instruments are often employed to clean up traumatic injury or to produce surgical wounds, it is absolutely necessary that the spores be at least rendered noninfectious; that is, if not absolutely killed, injured to the extent that they cannot cause disease when introduced into the wound. The inherent nature of the clostridia makes them one of the most important groups of bacteria to be considered in the disinfection of instruments. The conditions in the tissues following trauma, whether produced by an accident or by a surgical instrument, are ideal for the development of the anaerobes.

Another organism of great importance that has not been properly considered in testing these mercurials is Mycobacterium tuberculosis. Many obstacles accompany the use of this organism as a test agent. Cohn [65] enumerated these difficulties in 1933, and the slight

64. Walters, A. L.: The Specificity of Disinfectants and Its Bearing on Their Standardization, Am. J. Pub. Health **7**: 1030, 1917.

65. Cohn, M. L.: The Antiseptic Effect on Tubercle Bacilli of Certain Recently Advocated Compounds, J. Bact. **27**: 517, 1934.

advancement of knowledge since that time concerning the methods of cultivation does not make a thorough study of the germicidal action of the mercurials on Mycobacterium tuberculosis seem feasible at this time. It may be suspected that since evidence shows this organism to be less resistant to other chemical and physical agents than the clostridia, one might suspect that should these organic mercurials be germicidal for the spores of anaerobes they would be effective germicides for Mycobacterium tuberculosis. It may well be that the reason Clostridium tetani, septique or novyi has not been used in testing the organic mercurials is that most of the manufacturers producing these chemicals also manufacture biologicals or products for intravenous or intramuscular injection and hesitate to bring pathogenic anaerobes into their laboratories. However, some of these same companies produce toxoids and antitoxin with these bacteria. Very few laboratories are properly equipped to carry out mass experiments with these organisms. Many of the obstacles that apply to working with Mycobacterium tuberculosis also apply to the sporulating anaerobes.

In casting about for the proper species of the pathogenic clostridia to use in these experiments, I took several factors into consideration. A search of the literature does not reveal a very large number of cases of anaerobic infection traceable directly to contaminated or improperly sterilized instruments. Many laboratories are not properly equipped to make anaerobic examinations, and this is especially true of laboratories in rual communities and small hospitals with limited trained personnel. It is in these places that chemical sterilization for minor surgery is more often resorted to. It is also from such places that the medical personel is less likely to report its observations in the literature. The human element probably plays a far greater role in the absence of such reports in medical publications. An infection resulting from the use of an infected instrument may be indicative of carelessness and neglect. Probably many such cases are not reported in the literature. Wounds in which anaerobic infections develop are frequently the result of accidents, in which case the infection cannot be definitely attributed to the use of nonsterile instruments.

Nye and Mallory [40] report one of the most significant instances of anaerobic infection traceable directly to faulty sterilization of instruments. They report a case in which the surgical records show that an operation was performed on a man with infected (Cl. welchii) gunshot wounds. After the operation, the instruments were scrubbed with soap, scalded and put away in the cabinets as was the usual routine. Eight days later a tumor was removed from the scapula of another patient and two days later a patient was operated on for appendicitis. These latter patients developed Cl. welchii infections and died. Two other patients operated on at about the same time developed typical welchii infections and recovered. Sterility tests conducted on all the surgical materials revealed the Bard-Parker blades from the instrument case to be heavily contaminated with Cl. welchii. This report is important from various points of view, one of which is the method used for sterilization of the instruments. This method was as follows: As a matter of operating room routine after an operation, all scalpels, scissors and Bard-Parker blades were washed thoroughly with hot water and soap, rinsed in scalding water and then stored away in the instrument cases. Before being used again they were placed in a "sterilizing" solution (70 per cent alcohol) for from five to twenty minutes. Subsequent tests by Nye and Mallory with instruments purposely contaminated with the strain of organisms which caused the deaths mentioned demonstrated that immersion in alcohol (70 per cent) for one hour would not kill these bacteria. Another important point is that thorough mechanical cleansing with soap and water and then scalding still leaves enough organisms or spores to cause infection and death. A similar alcohol technic is being used by some surgeons, especially for very delicate cutting instruments which they dare not heat or boil. Much false confidence is placed in alcohol as a means of disinfection of syringes, needles and other commonly used apparatus. The use of alcohol for the sterilization of instruments is still being advocated by some surgeons.[66]

Orr,[67] in reviewing twenty-one cases of anaerobic infections of clean amputations, states: "Because of

66. Lawrence, G. P.: The Sterilization of Instruments in the Field, Mil. Surgeon **75**: 389, 1934.
67. Orr, T. G.: Gas Bacillus Infection Following Clean Amputations, Am. J. Surg. **25**: 113, 1934.

the great prevalence of gas producing organisms, the possibility of such infections should be kept in mind."

Millar [68] reviews 607 cases of gas gangrene in civil life and gives as the predisposing cause of seventy-two of these surgical operations and injections; the cause of 267 was not known.

Gangrenous infections frequently follow surgical procedures. A great many of these cases follow either gangrenous appendicitis or cholecystitis or an operation in which the field is contaminated with fecal contents. The usual site of infection is the tissue surrounding the incision, according to Eliason, Erb and Gilbert.[69] These facts would not be pertinent if it were not that instruments from such operations will be used on other patients and, unless the method of sterilization is effective, may lead to infection.

Zeissler,[70] writing concerning infection by anaerobic bacteria, gives the following incidence of the pathogenic anaerobes in 193 samples of soil: Cl. welchii was found in 100 per cent of the samples, Cl. oedematiens was found in 64 per cent of the samples, Cl. tetani was found in 27 per cent of the samples and Cl. septique was found in 8 per cent of the samples.

The report of the committee on anaerobic bacteria and their infections [71] firmly establishes the importance of this group of organisms as agents of infection, and this report brings to date the literature on the clostridia.

Nine postoperative cases of tetanus were reported in New South Wales in 1930 to 1933.[72] All nine cases followed abdominal operations and the examinations of the intestinal contents failed to reveal the organisms. Cl. tetani was isolated from the wound tissues from one of the four cases from which cultures were taken. Morgan is of the opinion that the catgut was responsible in most of the cases, for he states that five different brands of catgut were used in the nine operations. In

68. Millar, W. M.: Gas Gangrene in Civil Life, Surg., Gynec. & Obst. **54**: 232, 1932.

69. Eliason, E. L.; Erb, W. H., and Gilbert, P. D.: The Clostridium Welchii and Associated Organisms, Surg., Gynec. & Obst. **64**: 1005-1014, 1937.

70. Zeissler, J.: Infektionen durch anaerobe Bacillen, Klin. Wchnschr. **13**: 825, 1934.

71. Medical Research Committee of Great Britain: Report on the Anaerobic Infections of Wounds and the Bacteriological and Serological Problems Arising Therefrom, Special Report Series No. **39**, 88, 1919.

72. Morgan, E. L.: Postoperative Tetanus in New South Wales During the Years 1930-1933: Report of the Director-General of Public Health, New South Wales, 111, 1933 (printed, 1935).

seven instances biniodide of mercury was relied on to effect sterility of the catgut and culture tests confirmed the observations of other investigators as to its ineffectiveness. This is only another instance in which it is shown that a mercurial compound is not capable of sterilizing catgut. In these instances it was soaked as long as three weeks in 1 : 500 biniodide of mercury and then was not sterile on testing. Although the author states that it seems that the catgut was the source of infection in these cases, the instruments are not completely exonerated in at least two of the cases. He states that after the first tetanus operation all the instruments with the exception of knives were boiled for twenty minutes after the operation. The knives were washed thoroughly and placed in pure lysol. Mackie (1928) has shown this chemical to be inefficient in destroying tetanus spores. The same syringe was used on both these patients to give hypodermic injections following the operations. The syringes and needles were "sterilized" with alcohol between injections.

EXPERIMENTAL STUDIES

METHODS USED TO OBTAIN SPORES FOR THE EXPERIMENTAL WORK

It was found that a large yield of Cl. welchii spores could be obtained consistently by inoculating the surface of a fermented infusion agar [73] slant with a forty-eight hour infusion broth culture and then incubating four days in a Brown [24] anaerobe jar.

Cl. septique and Cl. tetani were grown on 1.5 per cent infusion agar, containing 1 per cent peptone and 0.5 per cent sodium chloride to which was added 0.5 cc. of sterile defibrinated blood per 5 cc. of agar (previously melted and cooled to from 45 to 50 C.). When large amounts of spore suspensions were needed large slants were made by layering blood agar over plain agar. The slants were innoculated from forty-eight hour cultures in infusion broth and were incubated in the Brown jar, as described, for four days. Microscopic examinations and culture after heating showed an abundance of mature spores.

73. This is 1.5 per cent agar made from an infusion which had been fermented by Bacterium coli before the addition of the salt, peptone, agar or the adjustment of *p*H. This agar was adjusted to *p*H 7.6—7.8 before being sterilized and had a reaction of *p*H 7.3 after sterilization.

For Cl. sporogenes and Bacillus anthracis infusion agar was used.

No attempt was made to get a suspension of spores in which there were no vegetative forms. This could have been accomplished by heating to 80 C. for fifteen minutes as suggested by Brown.[24] Although not killed by this heating, the spores might have been injured to the extent that the mercurials would have been able to destroy them. This would not have simulated actual conditions; therefore, cultural technic and microscopic examinations were depended on to insure a sufficient number of spores for the experiments. The heating technic was used in every case on a small amount of the spore suspension, before the experiments, to show whether or not viable spores were present.

ACTUAL PROCEDURE

The description of the technic using the Judd and Simon tube can best be given step by step.

1. The tubes are plugged and sterilized in the hot air oven. Test tubes with the same diameter as these tubes should be plugged with a gauze-covered divided plug, shown in *D* in the illustration on page 21, and sterilized at the same time. These plugs are to be used after the tube has been cut off and are of this type so that the divided ends may be folded down and fastened with a rubber band to prevent their being drawn into the tubes during centrifugation.

2. The spore suspensions are made and standardized. Spores are produced by methods described earlier and washed four times to remove any toxin or extraneous organic matter present. When clumps are present it is probable that the organisms in the center of the mass are protected from the action of the mercurial. To remove clumps the suspension is filtered through glass wool. This may be conveniently done by constructing an Allihin tube as suggested by Abbott.[11] This tube may be made by removing the centrifuge cone end from one of the Judd and Simon tubes by cracking it off with a hot wire and then inserting fine glass wool to make a filter layer about three-fourths inch thick. A cotton plug is inserted at each open end and the filter sterilized in the hot air over. In use, the cotton plug is removed from the small end and the tube inserted in a sterile test tube. The suspension is filtered and in addition to breaking up clumps any particles of agar present are removed. The suspension is standardized by the Hopkins tube method (Hopkins, 1931). The stock suspensions were made so that 10 cc. contained 0.25 cc. of sediment in a Hopkins tube when the revolutions per minute were 2,000. This particular amount was decided on because it was found that a slight tur-

bidity would remain after the repeated washing if the sediment were suspended in 0.4 cc. of sterile distilled water. The presence of a sufficient number of viable spores can be shown by plating dilutions of the suspension which have been heated to 80 C. for fifteen minutes. (For the anaerobes used in the following experiments the average number of viable spores used was 330,000,000 per cubic centimeter of the stock suspension).

3. Forty cc. of the mercurial to be tested is placed in the Judd and Simon tube by means of a 50 cc. syringe. An excellent device for filling these tubes is a discarded syringe, like those used by some biological houses as a container for serum of various kinds. Since these are easily obtained, it is possible to have one for each solution to be tested. Either a long needle of large bore or a small piece of capillary tubing may be attached. Care should be taken that this needle or tube passes all the way into the Judd and Simon tube past the constriction so that there will be no air trapped to cause backflow and loss of the chemical being tested.

4. After the mercurial is placed in the tube, 1 cc. of the stock spore suspension is introduced by means of a capillary pipet. The time is noted and the tube sealed off in the flame. One must seal the tip off as close to the body of the tube as possible, otherwise a dead end air trap is formed which will not wet properly. The correct method is shown in *B*, and the improper way at *B'* in the illustration (p. 21).

5. Since apparently none of the mercurials tested in the earlier work were capable of killing the spores on short contact, it was necessary to have a machine to rotate the tubes satisfactorily over long periods of time. An off-center cam arrangement was made so that when the tubes were placed on it horizontally they would be entirely wetted in going through each cycle. The dotted lines in *C* in the illustration show the extremes reached by the meniscus in traveling this cycle. The speed of the motor was set so that eighty complete cycles per minute were made. This was fast enough so that there was no chance for drying in any section of the tube. The tubes were rotated for twenty-three hours. About thirty minutes was required to innoculate from eight to twelve tubes and seal them in the flame before they were placed on the machine. Thirty minutes was required for the first centrifuging so that a total of twenty-four hours elapsed from the time the mercurial came in contact with the spores until it was removed.

6. After the tubes have been centrifuged and removed from the rocking machine a filed line was made just above the label, as shown in *B*. A hot curved nichrome wire is applied and the tube is cracked completely around. The top does not fly off but can be lifted away by hand, and the sterile plug already described is inserted. With the less pathogenic organisms the mercurial may be decanted, but with some of the organisms

a better technic is to remove the mercurial with a sterile 50 cc. volumetric pipet. All the mercurial but about 0.6 cc. can be decanted or pipetted off without disturbing the sediment. The remaining 0.6 cc. is thoroughly mixed with the sediment by means of a pipet and about 0.2 cc. is removed for animal inoculation. One loop of the spore-mercurial mixture is planted in an appropriate medium and incubated. Forty cc. of sterile distilled water is added and the organisms are resuspended by washing up and down several times with a very fine capillary pipet. The divided plug is replaced, fastened down by means of rubber bands and the tube centrifuged. This process is repeated three times, making a total of four washings. Although there is practically no turbidity in the final washing, when the sediment is diluted to 0.4 cc. a slight turbidity will remain, as stated in step 2.

7. The procedures from this point are dependent entirely on the organism used and must be taken up individually. The technic in general, however, has been to inoculate the proper recovery medium; that is, a medium which is ideal for the development of the spores if they have not been killed; in addition, mice have been inoculated to see whether or not the spores have been rendered noninfectious if not killed. The results of the experiments together with explanatory notes on special points to be observed with the individual bacteria follow. The results are given as far as possible in tabulated form in part I. In most cases the protocols were repeated twice, making three sets of experiments (table 2, p. 24).

EXPERIMENTS WITH CLOSTRIDIUM TETANI

The 4 mm. loop of mercurial spore mixture in step 6 (actual procedure) was planted into cooked meat medium under petrolatum. The 0.2 cc. of mercurial spore suspension was divided and 0.1 cc. mixed with 0.1 cc. of 1:30 lactic acid. One 4 mm. loop of this mixture was transferred as described to cooked meat.

After the fourth and final washing of the mercury-treated spores (step 6) the sediment in the bottom of the centrifuge tube was diluted to 0.4 cc. and the spores were resuspended with a capillary pipet; 0.1 cc. of these washed spores were mixed with 0.1 cc. of 1:20 lactic acid. One loop of this mixture as well as one loop of the washed spore suspension without lactic acid was planted in cooked meat.

White mice weighing 20 Gm. were injected intramuscularly in the thigh with the four spore suspensions; that is, the treated spores in the mercurial, the treated spores in the mercurial with lactic acid, the treated

spores washed four times and the treated spores washed four times plus lactic acid. It was found that the tetanus spores survived the twenty-four hour treatment in every instance and were still capable of causing death or producing tetanus. It was also possible to cultivate the spores in cooked meat medium.

Both chemical and organism controls were made. An approximately equivalent number of washed spores from the untreated spore suspension was injected, and 0.1 cc. of the mercurial with and without the addition of lactic acid was also injected. In no instance did this amount of mercurial cause death and no external lesions developed at the sight of injection. In every instance the animal injected with the spore control died in one or two days.

After death the mice were examined and a scraping and a cut were made directly through the muscle at the site of injection; this was done with a sterile detached surgeon's blade held in a previously sterilized and flamed hemostat. The blade was dropped into cooked meat and incubated. In every case Clostridium tetani was recovered.

In some instances the mice did not die, in which case they were held for two months and watched for signs of tetanus. Usually the leg in which they were injected became stiff in from three to five days. In some of the cases in which recovery occurred the stage of prostration had been reached; usually the only symptom was a stiff leg with possibly the involvement of the tail, which became stiff and erect and turned toward the side injected. In some cases the whole musculature of the side injected became involved and the mouse assumed the shape of a comma owing to the flexing of the spine.

EXPERIMENT WITH TINCTURES

Aqueous alcoholic acetone solutions, in the form of tinctures, of most of the mercurials are available. Since it is possible that the solvent might assist the penetration of the spore or dissolve the spore membrane so that the mercurial might be able to kill the organism, it was considered necessary to test the tincture solutions. Most of the tinctures contain 50 per cent ethyl alcohol, 40 per cent distilled water and 10 per cent acetone (all percentages are by volume).

The same technic was used for this experiment as was used in the previous ones with Clostridium tetani. The results differ only slightly from those obtained with the aqueous solutions. The spores were not killed in any instance.

EXPERIMENTS WITH CLOSTRIDIUM SEPTIQUE

The technic used for Cl. septique was the same as that described for Cl. tetani with one or two exceptions. The treatment of the organisms was the same as that given in the section Actual Procedure. The material was prepared and inoculated in the same manner as with Cl. tetani, and the medium used for recovery was cooked meat. The mice were autopsied in a different way. Whereas the organisms had been recovered from the site of injection with Cl. tetani, cultures of the heart blood were made from those animals dying after injections with Cl. septique. These organisms could also be found in smears made from the surface of the liver. Typical long filaments could be found in the dark red exudate in the peritoneal cavity. Little pathologic change was to be seen when the animals with tetanus were opened but the mice infected with Cl. septique presented a very characteristic change. These changes are described very accurately by Topley and Wilson,[14] page 680, and excellent color illustrations may be seen in the report of the Medical Research Committee on anaerobic infections (1919). Photographs of mice dying from tetanus and also Cl. welchii infections are given there for comparison.

Not one of the mercurials was able to destroy the spores of Cl. septique in every instance. It will be noted that all the mice dying from the septique infection died in either one or two days. It was possible to recognize those mice in which infection was developing before death. The leg and foot on the injected side assumed a purple color.

Mice injected with the untreated organism controls died in from twelve to twenty-four hours. Mercurial control mice showed no ill effects of their injection.

EXPERIMENTS WITH CLOSTRIDIUM WELCHII

The same treatment was given the spores of Cl. welchii as was outlined under actual procedure for Cl. tetani. The mercurial spore suspension was planted

into milk under petrolatum seal. The series of treated spore suspensions was mixed with lactic acid and injected as with the two preceding organisms. Untreated washed spore-suspensions were injected as controls, and the usual mercurial controls were also injected.

Although characteristic growth was obtained within forty-eight hours in twenty-four of the forty cultural tests, none of the corresponding mice died. The control mice injected with washed spores showed no signs of infection. It was decided that the strain was not virulent and another strain, which had been secured from one of the commercial houses, was used. The same results were obtained. A virulent strain used for the production of welchii toxin in a biologic house was tried, and still the washed spores failed to infect the mice even though lactic acid was included. One more trial was made. Some mice were injected with lactic acid and washed spores from a freshly isolated strain. This strain had killed mice when 0.1 cc. of a twenty-four hour broth culture was injected. No deaths resulted when the spores were washed twice.

These animal experiments had been somewhat disappointing but when the cultural tests were studied several interesting facts were noted.

From examination of the cultural results it appeared that several of the mercurials could be depended on to kill the spores of Cl. welchii. The question then arose whether the organisms could be recovered with the aid of some neutralizer. Making use of the extra 0.2 cc. amounts of treated organisms left over from the experiment recorded previously, another experiment was made. Part of the spores were allowed to remain in 1 per cent sodium thiosulfate for one half hour, then were washed with water. These organisms were then planted into milk and the organisms were recovered in every instance.

In several cases the time required for the stormy fermentation in the milk was cut in half. Two cases in which bacteria had apparently been killed grew out after the neutralization of the mercury. One of these required twenty-seven days, substantiating the statement made earlier that in experiments in which organisms are treated with disinfectants a comparatively short incubation period of from forty-eight hours to seven days is insufficient.

EXPERIMENTS WITH BACILLUS ANTHRACIS

Many of the earlier workers employed the spores of B. anthracis as a test organism. These were the first spores with which it was shown that mercurials were not always germicidal but exerted a very strong inhibitory action. These spores have been included in the present work principally for comparison.

The technic used for B. anthracis was practically the same as that described for the anaerobes. The recovery medium was broth containing serum as desecribed earlier in this work for use with spores of this organism. In addition, agar slants were streaked with the treated spores, since some workers have reported more positive results with B. anthracis when a solid medium was used.

The mice were injected subcutaneously rather than intramuscularly. Heart blood cultures were made from the mice dying after injection with the treated spores. In every instance in which a mouse died B. anthracis was recovered.

Since it appears that some of the mercurials were germicidal in certain instances, a neutralizer was employed. Some of the spores were allowed to remain for thirty minutes in 1 per cent sodium thiosulfate, and after the treatment with this neutralizer it was possible to recover anthrax in every instance by one of the methods used.

A comparative study of the results with B. anthracis indicates that none of the mercurials were capable of killing these spores in every instance in twenty-four hours under the conditions of testing.

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Printed and Published in the United States of America

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